

AMENDMENTS TO THE CLAIMS

Claim 1 (original) A composition for the prevention or control of coccidiosis comprising viable wild type sporulated oocysts of at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains at least about 10,000 oocysts per milliliter and less than about 0.8% by weight of alkali metal dichromate.

Claim 2 (original) A composition as set forth in claim 1 wherein the composition contains less than about 0.6% by weight of alkali metal dichromate.

Claim 3 (original) A composition as set forth in claim 2 wherein the composition contains less than about 0.4% by weight of alkali metal dichromate.

Claim 4 (original) A composition as set forth in claim 3 wherein the composition contains less than about 0.2% by weight of alkali metal dichromate.

Claim 5 (original) A composition as set forth in claim 4 wherein the composition contains less than about 0.1% by weight of alkali metal dichromate.

Claim 6 (original) A composition as set forth in claim 1 wherein said composition is characterized as substantially free of alkali metal dichromate.

Claim 7 (original) A composition as set forth in claim 1 wherein said composition contains less than about 0.3% by weight of dichromate ion.

Claim 8 (original) A composition as set forth in claim 7 wherein said composition contains less than about 0.15% by weight of hexavalent chromium.

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Claim 9 (original) A composition for the prevention or control of coccidiosis comprising viable wild type sporulated oocysts of at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate.

Claim 10 (original) A composition for the prevention or control of coccidiosis comprising viable wild type sporulated oocysts of at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains less than about  $5.0 \times 10^{-3}$   $\mu\text{g}$  of alkali metal dichromate per oocyst.

Claim 11 (original) A composition as set forth in claim 10 wherein said composition is sterile and contains less than about  $3.8 \times 10^{-3}$   $\mu\text{g}$  of alkali metal dichromate per oocyst.

Claim 12 (original) composition as set forth in of claim 11 wherein said composition is sterile and contains less than about  $1.3 \times 10^{-3}$   $\mu\text{g}$  of alkali metal dichromate per oocyst.

Claim 13 (original) A composition as set forth in of claim 12 wherein said composition is sterile and contains less than about  $6.3 \times 10^{-5}$   $\mu\text{g}$  of alkali metal dichromate per oocyst.

Claim 14 (original) A composition as set forth in claim 1, further comprising a diluent.

Claim 15 (original) A composition as set forth in claim 14, wherein the diluent comprises water.

Claim 16 (original) A composition as set forth in claim 15, wherein the aqueous diluent comprises 0.5X phosphate buffered saline.

Claim 17 (original) A composition as set forth in claim 16 further comprising a buffer.

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Claim 18 (original) A composition as set forth in claim 17, wherein said buffer is selected from the group consisting of phosphate buffer, bicarbonate buffer, citric acid and tris buffers.

Claim 19 (original) A composition as set forth in claim 17, wherein said buffer controls pH between about 7.0 and about 7.8

Claim 20 (original) A composition as set forth in claim 14, further comprising a bactericide.

Claim 21 (original) A composition as set forth in claim 20, wherein said bactericide is selected from the group consisting of potassium perchlorate, sodium hypochlorite, hydrochlorous acid, sodium hydroxide and antibiotics.

Claim 22 (original) A composition as set forth in of claim 21, wherein said composition contains from about 0.1 to about 0.75 wt% potassium perchlorate, and/or from about 0.001 to about 0.01 wt% sodium hypochlorite, and/or from about 1 to about 5 ppm hydrochlorous acid, and/or from about 0.5 to about 1.5 mM sodium hydroxide and/or from about 20 to about 30 µg/ml gentamicin, in the final composition.

Claim 23 (original) A composition as set forth in claim 14, further comprising a composition that ameliorates a decline in post-challenge performance.

Claim 24 (original) A composition as set forth in claim 23, wherein said composition is selected from the group consisting of Alum, Freund's adjuvant, calcium phosphate, beryllium hydroxide, dimethyl dioctadecyl ammonium bromide, saponins, polyanions, Quil A, inulin, lipopolysaccharide endotoxins, liposomes, lysolecithins, zymosan, propionibacteria,

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mycobacteria, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interferon- $\alpha$ , interferon- $\gamma$ , and granulocyte-colony stimulating factor.

Claim 25 (original) A composition as set forth in claim 23, wherein said composition is selected from the group consisting of cytokines, growth factors, chemokines, mitogens and adjuvants.

Claim 26 (original) A composition as set forth in claim 25, wherein said composition comprises *Propionibacterium acnes*.

Claim 27 (original) A composition as set forth in claim 26, wherein said composition contains at least about 3.0 milligrams (dry weight) of *P. acnes* per milliliter.

Claim 28 (original) A composition as set forth in claim 26, wherein said composition contains at least about 30 milligrams (dry weight) of *P. acnes* per milliliter.

Claim 29 (original) A composition as set forth in claim 1 comprising:

viable sporulated oocysts of at least one species of protozoa known to cause coccidiosis,

a diluent,

a buffer, and

a bactericide,

wherein said composition contains about 10,000 oocysts per milliliter and less than about 0.8% weight to volume of alkali metal dichromate.

Claim 30 (original) A composition as set forth in claim 29, further comprising a composition that ameliorates a decline in post-challenge performance.

Claim 31 (original) A method for producing a composition for the prevention or control of coccidiosis comprising:

collecting manure from host animals wherein said manure contains oocysts known to cause coccidiosis;

diluting said manure in an aqueous medium to create a slurry;

separating unwanted fecal matter from said slurry and collecting the aqueous fraction containing oocysts;

subjecting said aqueous fraction to solid/liquid phase centrifugal-based separation and collecting the solid phase;

combining a dense aqueous liquid with said collected solid phase wherein said dense liquid has a density greater than about 1.09 g/ml and wherein the oocysts are buoyant;

subjecting the combination of said dense aqueous liquid and collected solid phase to centrifugation and collecting the dense liquid fraction containing oocysts;

diluting said dense liquid fraction to a specific gravity wherein the oocysts are no longer buoyant;

separating oocyst solids from said diluted liquid fraction by centrifugal-based separation and re-collecting the solid phase.

Claim 32 (original) A method as set forth in claim 31 further comprising:

diluting said re-collected solid phase in an aqueous sporulation medium;

sporulating said oocysts while in contact with said sporulation medium;

separating sporulated oocysts from said sporulation medium;

sterilizing said sporulated oocysts; and

diluting said sporulated oocysts to form a vaccine composition.

Claim 33 (original) A method of separating oocysts from a liquid suspension by the use of a hydrocyclone.

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Claim 34 (original) A method as set forth in claim 33 wherein the oocysts are collected in the underflow from the hydrocyclone.

Claim 35 (original) A method for isolating oocysts comprising:

collecting manure from host animals wherein said manure contains oocysts known to cause coccidiosis;

diluting said manure in an aqueous medium to create a slurry;

separating unwanted fecal matter from said slurry and collecting the aqueous fraction containing oocysts;

subjecting said aqueous fraction to solid/liquid phase centrifugal-based separation by means of a hydrocyclone.

Claim 36 (original) A method for isolating oocysts comprising:

collecting manure from host animals wherein said manure contains oocysts known to cause coccidiosis;

diluting said manure in an aqueous medium to create a slurry;

separating unwanted fecal matter from said slurry and collecting the aqueous fraction containing oocysts;

subjecting said aqueous fraction to solid/liquid phase centrifugal-based separation and collecting the solid phase;

combining a dense aqueous liquid with said collected solid phase wherein said dense liquid has a density greater than about 1.09 g/ml and wherein the oocysts are buoyant;

subjecting the combination of said dense aqueous liquid and collected solid phase to centrifugation and collecting the dense liquid fraction containing oocysts;

diluting said dense liquid fraction to a specific gravity wherein the oocysts are no longer buoyant;

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separating oocyst solids from said liquid phase by means of a hydrocyclone and re-collecting the solid phase.

Claim 37 (original) A method as set forth in claim 31 wherein said animals comprise the class Aves.

Claim 38 (original) A method as set forth in claim 37 wherein said slurry is created by mixing manure and water in relative proportions of from about 0.5 gallons to about 5 gallons of domestic water per the amount of manure obtained in about 3 days from about six animals comprising the class Aves.

Claim 39 (original) A method as set forth in claim 38 wherein said animals are chickens.

Claim 40 (original) A method as set forth in claim 31 wherein said separation of unwanted fecal matter comprises sieving.

Claim 41 (original) A method as set forth in claim 40 wherein said sieving is by the use of multiple-tier shaker screens.

Claim 42 (original) A method as set forth in claim 41 wherein said shaker screens comprise a 50-mesh screen and a 250-mesh screen.

Claim 43 (original) A method as set forth in claim 31 wherein said method is carried out at a temperature between about 4° C and about 30° C.

Claim 44 (original) A method as set forth in claim 43 wherein said sieving is carried out at a temperature between about 22° C and about 28° C.

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Claim 45 (original) A method as set forth in claim 44 wherein said sieving is carried out at about 25° C.

Claim 46 (original) A method as set forth in claim 31 wherein each of said centrifugal-based separations comprises the use of a centrifuge or a hydrocyclone.

Claim 47 (original) A method as set forth in claim 46 wherein said centrifugal-based separation comprises the use of a hydrocyclone.

Claim 48 (original) A method as set forth in claim 47 wherein said centrifugal-based separation comprises the use of a centrifuge.

Claim 49 (original) A method as set forth in claim 48 wherein said centrifuge is a bottle centrifuge.

Claim 50 (original) A method as set forth in claim 48 wherein said centrifuge is a continuous centrifuge.

Claim 51 (original) A method as set forth in claim 31 wherein said centrifugation is a bottle centrifuge.

Claim 52 (original) A method as set forth in claim 31 wherein said dense aqueous liquid comprises a solution of corn syrup or sodium chloride.

Claim 53 (original) A method as set forth in claim 31 wherein said aqueous solution has a density from about 1.07 g/ml to about 1.20 g/ml.

Claim 54 (original) A method as set forth in claim 53 wherein said aqueous solution has a density from about 1.08 g/ml to about 1.14 g/ml.

Claim 55 (original) A method as set forth in claim 54 wherein said aqueous solution has a density from about 1.09 g/ml to about 1.10 g/ml.

Claim 56 (original) A method for inducing sporulation of oocysts comprising:

introducing into an aqueous sporulation medium oocysts of at least one species of protozoa known to cause coccidiosis;

incubating said oocysts in said aqueous sporulation medium, thereby causing sporulation of oocysts; and

introducing an oxidizing agent into said medium at a rate sufficient to maintain the average dissolved oxygen content during sporulation at at least 30%.

Claim 57 (original) A method as set forth in claim 56 wherein said dissolved oxygen content is substantially maintained at at between about 30% and about 80% of saturation throughout the period of sporulation.

Claim 58 (original) A method as set forth in claim 57 wherein said dissolved oxygen content of the medium is substantially maintained at between about 40% and about 60% of saturation throughout the period of sporulation.

Claim 59 (original) A method as set forth in claim 58 wherein said dissolved oxygen content of the medium is substantially maintained at about 50% of saturation.

Claim 60 (original) A method as set forth in claim 56 wherein the alkali metal dichromate content of said sporulation medium is less than about 0.8% by weight during incubation of oocysts.

Claim 61 (original) A method as set forth in claim 56 comprising addition to said sporulation medium of an oxidizing agent having a standard reduction potential of at least about 0.5 V.

Claim 62 (original) A method as set forth in claim 61 comprising addition of both molecular oxygen and another oxidizing agent.

Claim 63 (original) A method as set forth in claim 62 wherein said oxidizing agent has a standard reduction potential of at least about 0.5 V.

Claim 64 (original) A method as set forth in claim 63 wherein said oxidizing agent is selected from the group consisting of an alkali metal hypochlorite, an alkali metal chlorite, an alkali metal chlorate, an alkali metal perchlorate, and an alkali metal permanganate.

Claim 65 (original) A method as set forth in claim 64 wherein said oxidizing agent comprises hypochlorite ions.

Claim 66 (original) A method as set forth in claim 64 wherein a sufficient amount of an alkali metal hypochlorite is added to achieve an alkali metal hypochlorite weight percent from about 0.001 weight percent to about 0.1 weight percent of the sporulation medium and oocysts combined, wherein said alkali metal hypochlorite is from about 1.0 % to about 10.0 % by volume.

Claim 67 (original) A method as set forth in claim 56 further comprising:  
separating sporulated oocysts from said sporulation medium;  
sterilizing sporulated oocysts by contacting said sporulated oocysts with a chemical disinfectant; and

storing said sporulated oocysts in a sterile diluent, said diluent containing less than about 0.8% by weight alkali metal dichromate.

Claim 68 (original) A method as set forth in claim 56 wherein said medium contains less than about 0.3% by weight dichromate ion during incubation of said oocysts.

Claim 69 (original) A method as set forth in claim 56 wherein said medium contains less than about 0.15% by weight hexavalent chromium during incubation of said oocysts.

Claim 70 (original) A method as set forth in claim 56 wherein said dissolved oxygen content is established by bubbling an oxygen-containing gas through said sporulation medium.

Claim 71 (original) A method as set forth in claim 70 wherein said oxygen-containing gas consists essentially of air.

Claim 72 (original) A method as set forth in claim 70 wherein said gas comprises commercially pure oxygen.

Claim 73 (original) A method as set forth in claim 56 further comprising maintaining the temperature from a temperature that substantially avoids freezing to about 45° C.

Claim 74 (original) A method as set forth in claim 73 wherein temperature is maintained from about 15° C to about 40° C.

Claim 75 (original) A method as set forth in claim 74 wherein temperature is maintained from about 20° C to about 30° C.

Claim 76 (original) A method as set forth in claim 75 wherein temperature is maintained at about 28° C.

Claim 77 (original) A method as set forth in claim 56 further comprising incubating the oocysts under said conditions from about 72 hours to about 120 hours.

Claim 78 (original) A method as set forth in claim 77 wherein the oocysts incubate from about 72 hours to about 96 hours.

Claim 79 (original) A method as set forth in claim 78 wherein the oocysts incubate for about 72 hours.

Claim 80 (original) A method as set forth in claim 56 further comprising controlling the pH of the sporulation medium.

Claim 81 (original) A method as set forth in claim 79 wherein the pH is controlled by the introduction of an acid or base to the sporulation medium.

Claim 82 (original) A method as set forth in claim 81 wherein the pH of the sporulation medium is controlled by alternatively adding sodium hydroxide and sulfuric acid to the sporulation medium.

Claim 83 (original) A method as set forth in claim 82 wherein the pH of the sporulation medium is controlled from about 7.2 to about 7.5.

Claim 84 (original) A method as set forth in claim 83 wherein the pH of the sporulation medium is controlled at about from 7.35 to about 7.45.

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Claim 85 (original) A method as set forth in claim 67 wherein said sporulated oocysts are separated from said sporulation medium by filtration or by centrifugal-based separation.

Claim 86 (original) A method as set forth in claim 85 wherein said sporulated oocysts are separated by filtration.

Claim 87 (original) A method as set forth in claim 86 wherein said sporulated oocysts are separated from the sporulation medium by tangential flow filtration.

Claim 88 (original) A method as set forth in claim 67 wherein said sterilization is achieved by adding a chemical disinfectant to sporulated oocysts separated from said sporulation medium.

Claim 89 (original) A method as set forth in claim 88 wherein said sterilization substantially eliminates microorganisms.

Claim 90 (original) A method as set forth in claim 89 wherein said microorganisms are selected from the group comprising infectious bursal disease virus and chicken anemia virus.

Claim 91 (original) A method as set forth in claim 88 wherein said sterilization is by a chemical disinfectant other than an alkali metal dichromate.

Claim 92 (original) A method as set forth in claim 88 wherein said chemical disinfectant comprises a solution of an alkali metal hypochlorite.

Claim 93 (original) A method as set forth in claim 92 wherein said chemical disinfectant comprises a solution of sodium hypochlorite.

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Claim 94 (original) A method as set forth in claim 93 wherein said solution used is at a concentration from about 1% to about 20% by volume of active chlorine.

Claim 95 (original) A method as set forth in claim 94 wherein said is at a concentration from about 5% to about 15% by volume of active chlorine.

Claim 96 (original) A method as set forth in claim 95 wherein said solution is at a concentration of about 10% by volume of active chlorine.

Claim 97 (original) A method as set forth in claim 93 wherein said sporulated oocysts are treated with said sodium hypochlorite from about 5 to about 25 minutes.

Claim 98 (original) A method as set forth in claim 97 wherein said sporulated oocysts are treated with sodium hypochlorite from about 8 to about 20 minutes.

Claim 99 (original) A method as set forth in claim 98 wherein said sporulated oocysts are treated with sodium hypochlorite for about 10 minutes.

Claim 100 (original) A method as set forth in 92 further comprising substantially separating said sodium hypochlorite from the sporulated oocysts by filtration.

Claim 101 (original) A method as set forth in claim 100 wherein said filtration is by means of tangential flow filtration.

Claim 102 (original) A method for inducing sporulation of oocysts comprising:  
introducing into an aqueous sporulation medium oocysts of at least one species of protozoa known to cause coccidiosis;

incubating said oocysts in said aqueous sporulation medium, thereby causing sporulation of oocysts; and

introducing an oxidizing agent having a standard reduction potential of at least about 0.5 V into said medium at a rate sufficient to maintain the oxidation potential of said medium equivalent to the oxidation potential of a medium containing dissolved molecular oxygen in concentration of at least 30% of saturation during sporulation;

said medium containing less than about 0.8% by weight alkali metal dichromate during incubation of said oocysts.

Claim 103 (original) A method for monitoring sporulation of oocysts comprising:

incubating viable oocysts in an aqueous sporulation medium; and

during incubation, monitoring said medium to detect a change in at least one of the following parameters:

- (i) dissolved oxygen content;
- (ii) pH;
- (iii) rate of introduction of oxidizing agent into said medium;
- (iv) flow rate of acid or base into said medium.

Claim 104 (original) A method as set forth in claim 103 wherein dissolved oxygen content of said medium is controlled by addition of molecular oxygen thereto, and monitoring sporulation comprises detecting a change in oxygen consumption as indicated by detection of a change in oxygen flow to the medium and/or a permanent or transient change in dissolved oxygen content.

Claim 105 (original) A method as set forth in claim 103 wherein pH of said medium is controlled by addition of acid or base thereto, and monitoring sporulation comprises detecting an increase in acid consumption as indicated by an increase in acid flow to the medium and/or a permanent or transient increase in pH.

Claim 106 (original) A method as set forth in claim 103 wherein the end point of sporulation is determined from substantial cessation of oxygen consumption or generation of alkalinity in the sporulation medium.

Claim 107 (original) A method as set forth in claim 106 wherein the end point is indicated by the substantial cessation of change in at least one of said parameters.

Claim 108 (original) A method as set forth in claim 106 wherein said sporulated oocysts are maintained in said medium under sporulation conditions for at least another 48 hours after the indicated end point of sporulation.

Claim 109 (original) A method as set forth in claim 103, wherein said change in dissolved oxygen content is a decrease.

Claim 110 (original) A method as set forth in claim 103, wherein said change in pH is an increase.

Claim 111 (original) A method as set forth in claim 110, wherein said increase in pH is at least 0.5 pH units.

Claim 112 (original) A method as set forth in claim 111, wherein said increase in pH is at least 0.25 pH units.

Claim 113 (currently amended) A kit for the prevention or control of coccidiosis comprising,

a composition containing, sterile, viable, sporulated oocysts of at least one species of protozoa known to cause coccidiosis, said composition containing less than about 0.8% by weight of alkali metal dichromate; and

instructions for administration of said composition to an animal.

Claim 114 (original) A kit as set forth in claim 113 containing less than about 0.3% by weight of dichromate ion.

Claim 115 (original) A kit as set forth in claim 113 containing less than about 0.15% by weight of hexavalent chromium.

Claim 116 (original) A kit as set forth in claim 113 characterized as substantially free of alkali metal dichromate.

Claim 117 (original) A kit as set forth in claim of 113, wherein said composition comprises the composition of claim 1.

Claim 118 (original) A kit as set forth in claim 113, further comprising:

a diluent, said diluent characterized as substantially free of alkali metal dichromate; and

instructions for mixing said diluent with said composition to form a mixture.

Claim 119 (original) A kit according to claim 118, wherein said diluent comprises a sterile diluent.

Claim 120 (original) A composition for the storage of sporulated oocysts comprising an aqueous diluent and a bactericide, said composition characterized as substantially free of alkali metal dichromate wherein said composition is characterized as having:

a diluent comprising 0.5X phosphate buffered saline;  
a pH from about 5.0 to about 8.0; and

wherein said bactericide is selected from the group consisting of an alkali metal perchlorate, an alkali metal hypochlorite, hydrochlorous acid, sodium hydroxide and antibiotics.

Claim 121 (original) A composition as set forth in claim 120 having a pH from about 7.0 to about 7.5.

Claim 122 (original) A composition as set forth in claim 120 wherein said bactericide comprises gentamicin.

Claim 123 (original) A composition as set forth in claim 120 further comprising an oxidizing agent.

Claim 124 (original) A composition as set forth in claim 120 characterized in that an oocyst population in contact with said composition remains at least about 60% viable for 13 weeks at about 25° C.

Claim 125 (original) A composition as set forth in claim 120 characterized in that an oocyst population in contact with said composition remains at least about 60% viable for 26 weeks at about 5°C.

Claim 126 (original) A composition as set forth in claim 120 characterized in that an oocyst population in contact with said composition decrease in viability no more than about 20% over a period of at least about 13 weeks at about 25°C.

Claim 127 (original) A composition as set forth in claim 120 characterized in that an oocyst population in contact with said composition decrease in viability no more than about 20% over a period of at least about 26 weeks at about 5°C.

Claim 128 (original) A composition as set forth in claim 120 further comprising a dye.

Claim 129 (original) A composition for the storage of sporulated oocysts comprising:

0.5X PBS; and

about 30 µg/ml gentamicin,

said composition characterized as substantially free of alkali metal dichromate, and wherein said composition is characterized in that oocysts in contact with said composition decrease in viability no more than about 20% over a period of at least about 26 weeks at about 5°C.

Claim 130 (original) A method for storing sporulated oocysts comprising contacting said sporulated oocysts with the composition of claim 120.

Claim 131 (original) A method as set forth in claim 130 further comprising storing said sporulated oocysts in contact with the composition of claim 120 at either about 25°C or about 5°C.

Claim 132 (original) A method as set forth in claim 130 wherein said population of sporulated oocysts is maintained at least 60% viable for 13 weeks at about 25°C.

Claim 133 (original) A method as set forth in claim 130 wherein said population of sporulated oocysts is maintained at least 60% viability for 26 weeks at about 5°C.

Claim 134 (original) A method as set forth in claim 130 wherein said method prevents a decrease in oocyst viability of greater than 20% over a period of at least 13 weeks at about 25°C.

Claim 135 (original) A method as set forth in claim 130 wherein said method prevents a decrease in viability of greater than 20% in a population of sporulated oocysts over a period of at least 26 weeks at about 5°C.

Claim 136 (original) A composition for the prevention or control of coccidiosis comprising viable wild type sporulated oocysts of at least one species of protozoa known to cause coccidiosis, said composition having been made by the method of claim 56, wherein said composition is characterized as substantially free of alkali metal dichromate.

Claim 137 (original) A composition as set forth in claim 136 wherein said composition comprises viable wild type sporulated oocysts from a species of *Eimeria* selected from the group consisting of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*.

Claim 138 (original) A composition as set forth in claim 137 wherein said composition comprises viable wild type sporulated oocysts of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*.

Claim 139 (original) A composition as set forth in claim 137 wherein said vaccine comprises viable wild type sporulated oocysts of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* in a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations.

Claim 140 (original) A composition as set forth in claim 137 wherein said composition comprising at least about  $1.25 \times 10^4$  viable wild type sporulated oocysts per milliliter.

Claim 141 (original) The composition as set forth in claim 137 wherein said composition is characterized as substantially free of added bactericide.

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Claim 142 (original) A composition as set forth in claim 137 comprising a composition which ameliorates a decrease in post-challenge performance.

Claim 143 (original) A composition as set forth in claim 142 wherein said composition comprises *Propionibacterium acnes*.